

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C07K 7/00, 14/00, 16/00, C12P 21/08</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 95/25119</b> <b>(43) International Publication Date:</b> 21 September 1995 (21.09.95)
<b>(21) International Application Number:</b> PCT/US95/03180 <b>(22) International Filing Date:</b> 16 March 1995 (16.03.95) <b>(30) Priority Data:</b> 08/214,347 16 March 1994 (16.03.94) US <b>(71) Applicant:</b> MALLINCKRODT MEDICAL, INC. [US/US]; 675 McDonnell Boulevard, P.O. Box 5840, St. Louis, MO 63134 (US). <b>(72) Inventors:</b> MILLER, Kathleen, M.; 13002 Montmartre Drive, Ferguson, MO 63141 (US). DE LA FOURNIERE, Lau- rence; 78, rue Tronchet, F-69006 Lyon (FR). <b>(74) Agents:</b> MCBRIDE, Thomas, P. et al.; Mallinckrodt Medical, Inc., 675 McDonnell Boulevard, P.O. Box 5840, St. Louis, MO 63134 (US).		<b>(81) Designated States:</b> CA, CZ, FI, HU, JP, MX, NO, PL, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> STABILIZATION OF PEPTIDES AND PROTEINS FOR RADIOPHARMACEUTICAL USE		
<b>(57) Abstract</b> <p>Surfactants alone or in combination with salts are used to stabilize radiolabeled peptides and proteins contained in diagnostic or therapeutic radiopharmaceutical compositions.</p>		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

**STABILIZATION OF PEPTIDES AND PROTEINS FOR  
RADIOPHARMACEUTICAL USE**

FIELD OF THE INVENTION

This invention relates generally to a novel use  
5 of surfactants alone or in combination with salts to  
stabilize radiolabeled peptides and proteins contained in  
diagnostic or therapeutic radiopharmaceutical compositions  
and, more particularly, to the use of one or more  
10 surfactants alone or in combination with one or more salts  
to stabilize radiolabeled peptides, polypeptides or  
proteins contained in diagnostic or therapeutic  
radiopharmaceutical compositions for diagnostic tissue  
imaging or therapeutic use.

BACKGROUND OF THE INVENTION

15 Scintigraphic imaging and similar radiographic  
techniques for visualizing tissues in vivo are finding  
ever-increasing applications in diagnostic procedures.  
Generally, scintigraphic procedures involve the preparation  
of radioactive agents which, upon introduction to a  
20 biological subject, become localized in the specific organ,  
tissue, or skeletal structure of choice. When so  
localized, traces, plots, or scintigraphic images depicting  
the in vivo distribution of radioactive agents can be  
generated from data collected by various radiation  
25 detectors, such as scintillation cameras. The distribution  
and corresponding relative intensity of the detected  
radioactive agent indicate the space occupied by the  
targeted tissue and may also indicate a presence of  
receptors, antigens, aberrations, pathological conditions,  
30 and the like.

Radioactive agents are also finding increasing applications in therapeutic procedures. As with scintigraphic procedures, the radioactive agents, upon introduction to a biological subject, become localized in the specific organ, tissue, or skeletal structure of choice. Emissions from the radionuclide deliver a therapeutic dose to the targeted tissue.

In general, depending on the type of radionuclide and the target organ or tissue of interest, the compositions of the radioactive agents comprise a radionuclide, a carrier agent designed to target the specific organ or tissue site, possibly various auxiliary agents, e.g. chelating agents, which affix the radionuclide to the carrier, and water or other delivery vehicles suitable for injection into, or aspiration by, the patient, such as physiological buffers, salts, and the like.

Over the years, there has been growing interest in preparing radiolabeled proteins such as macroaggregated albumin ("MAA"), human serum albumin ("HSA"), monoclonal antibodies, or monoclonal antibody fragments for the purpose of diagnosing and treating diseases, such as inflammation, deep vein thrombosis, or cancer.

Recently, the use of radiolabeled peptides for diagnostic and therapeutic applications has attracted much attention. One such radiolabeled peptide is derived from an octapeptide somatostatin analog known as octreotide and is described in U.S. Patent No. 4,395,403. Octreotide has a very high binding affinity to somatostatin receptors in a variety of human tumors. By linking octreotide to a suitable chelating agent capable of forming a complex with radionuclides, it has been possible to create radiolabeled octreotide which effectively images tumors having

somatostatin receptors. Somatostatin analogs containing chelating groups are described in greater detail in UK Patent Publication No. 2,225,579.

Despite the potential usefulness of radiolabeled  
5 peptides and proteins, it has been found that such radiolabeled compounds are extremely susceptible to radiolysis, caused by the radionuclide attached thereto for a label.

As used herein, the term radiolysis includes  
10 chemical decomposition of the peptide, polypeptide, or protein by the action of radiation emitting from the radionuclide attached for a label. This chemical decomposition can occur in the radiopharmaceutical compositions incubated at room temperature. Often, in the  
15 preparation of radiopharmaceutical compositions, these preparations require heating to form the desired product or autoclaving for sterilization. Either of these two processes may accelerate the decomposition of the radiolabeled compounds by the action of radiolysis.

20 To inhibit or prevent radiolysis, experts have suggested adding a stabilizer such as HSA to the composition (e.g., R.A.J. Kishore, et al., "Autoradiolysis of Iodinated Monoclonal Antibody Preparations," Int. J. Radiat. Appl. Instrum., Part B, Vol. 13, No. 4, pp. 457-459  
25 (1986) and European Patent Application having International Publication Number WO91/04057), keeping the radiopharmaceutical composition frozen between preparation and administration (e.g., R.L. Wahl, et al., "Inhibition of Autoradiolysis of Radiolabeled Monoclonal Antibodies by  
30 Cryopreservation," J. Nuc. Med., Vol. 31, No. 1, pp. 84-89 (1990)), or storing a radiolabeled biological molecule in contact with an ion exchange resin (European Patent Application 0 513 510 A1). These techniques for preventing

radiolysis are often not effective or practical when used with many radiolabeled peptides and proteins.

Surfactants have been shown to alter the reactivity of radiolytically generated radicals (e.g.  $e^{-1}$  (aq), OH, H) towards substrates such as benzene (J. H. Fendler, et al., "Radiation Chemistry of Aqueous Micellar Systems", Report, RRL-3238-364, 12 pp., Avail, Dep. NTIS), pyrimidines (J.H. Fendler, et al., "Radiolysis of Pyrimidines in Aqueous Solutions", J. Chem. Soc., Faraday Trans. 1, Vol. 70, No. 7, pp. 1171-9, 1974) and dimethyl viologen cations (M.A.J. Rodgers, D. C. Foyt, and Z. A. Zimek, "The Effect of Surfactant Micelles on the Reaction Between Hydrated Electrons and Dimethyl Viologen", Radiat. Res., Vol. 75, No. 2, p. 296-304, 1978). Cationic, nonionic, and anionic surfactants have been used, at concentrations both below and above the critical micelle concentration. Reaction rates have been observed to decrease, increase, or remain unchanged, depending on the type of surfactant, the substrate used, and the free radical produced by gamma radiolysis.

Surfactants and in particular polyoxyethylene (20) sorbitan monooleate have likewise been said to stabilize dilute protein solutions (e.g., Dr. W. R. Ashford and Dr. S. Landi: "Stabilizing Properties of Tween® 80 in Dilute Protein Solutions" Bull. Parent. Drug Assoc., Vol. 20, pp. 74-81, 1966). Ashford, et al. suggests that such solution stabilization could be applicable to radiolabeled proteins as directed to the prevention of adsorption to glass since adsorption results in a loss of potency of the particular protein solution. Surfactants have also been taught as stabilizers of proteins against denaturing, thus maintaining, for example, their enzymatic activity or their solubility at an air/liquid interface (Y. J. Wang and M. A.

Hanson, "Parenteral Formulations of Proteins and Peptides: Stability and Stabilizers", J. Parent. Sci. Tech., Vol. 42, pp. S4-S26, 1988, and references therein). The use of surfactants as stabilizers of large molecules such as radiolabeled protein, polypeptides, or peptides to avoid radiolytic decomposition has not been previously taught.

From the foregoing, it will be appreciated that what is needed in this particular art field are stabilizers for radiolabeled peptides and proteins in a pharmaceutically effective concentration. Thus, it would be a significant advancement in this art field to provide stabilizing agents which substantially inhibit radiolysis of such radiolabeled peptides, polypeptides, and proteins.

Such stabilizers for substantially inhibiting peptide, polypeptide, or protein radiolysis are thereby disclosed and claimed herein.

#### SUMMARY OF THE INVENTION

The present invention overcomes and prevents the problem of radiolytic decomposition of radiolabeled peptides, polypeptides or proteins that are contained in diagnostic or therapeutic radiopharmaceutical compositions. The present invention discloses the use of surfactants, used alone or in combination with one or more salts, to stabilize radiolabeled peptides, polypeptides or proteins and thereby avoid radiolytic decomposition thereof. These stabilized compounds may be used for the diagnostic imaging of targeted tissues or for therapeutic treatment.

In diagnostic imaging localization, a radiolabeled compound must be easily detectable and highly selective. High selectivity, which is essential in these

compounds, means that the diagnostic compound, after having been introduced into the body, accumulates to a greater degree in the target tissue or tissues, e.g. a malignant tumor, than in surrounding tissues. In using peptides, polypeptides or proteins as carrier agents in radiolabeled compounds, the specific high selectivity of the particular carrier agent used provides for the strong accumulation of the diagnostic compound in the target tissue or tissues, such as, for example, in tumors compared with the concentration thereof in non-target tissues.

For therapeutic treatment, the radiolabeled peptide, polypeptide, or protein compound is constructed using high energy beta or alpha emitting isotopes rather than the pure gamma emitters customarily used for diagnostic purposes. These therapeutic agents must have the same high selectivity property as the diagnostic agents to achieve therapeutic treatment of target tissues, such as malignant tumors.

A problem commonly encountered in radiolabeling peptides, polypeptides and proteins overcome by the present invention is that radiolabeled peptides, polypeptides and proteins are highly susceptible to decomposition from radiolysis. This radiolytic decomposition can be accelerated if the radiolabeled preparation is heated or autoclaved.

The present invention overcomes this problem of radiolysis by stabilizing the radiolabeled peptide, polypeptide or protein compound in the diagnostic or therapeutic formulation through the use of one or more suitable surfactants used alone or in combination with one or more suitable salts, to prevent radiolytic decomposition and to preserve the specificity of the compound.



The above-described present invention makes radiolabeled peptides, polypeptides and proteins previously viewed as being difficult to stabilize very attractive for diagnostic purposes as well as for radiotherapy. This method of stabilization of radiolabeled compounds is useful with any radionuclide favorable for imaging or therapy. For diagnostic imaging purposes, the most suitable radionuclides include but are not limited to indium-111, technetium-99m, iodine-123, gallium-67, and copper-62. Suitable radionuclides for radiotherapy include but are not limited to rhenium-186, rhenium-188, copper-67, iodine-131, copper-67, yttrium-90, dysprosium-165, samarium-153, holmium-166, strontium-89, rhodium-105, and cobalt-60.

Radiolabeling of peptides and proteins can be achieved using various methods known in the art. For example, peptides can be labeled through use of a bifunctional chelate, direct labeling, or covalent binding to a specific functional group of an amino acid side chain. The use of a bifunctional chelate involves covalent attachment of a chelate, which complexes with the radionuclide, to the peptide or protein. Possible bifunctional chelates include diethylene triaminepentaacetic acid (DTPA) and triamine thiolate ( $N_3S$ ) ligands. DTPA may be attached to the peptide or protein by the dicyclic dianhydride method described in U.S. Patent No. 4,479,930, which is incorporated herein by reference.  $N_3S$ -type ligands may be attached to the peptide or protein by the methods described in U.S. Patent No. 4,965,392 incorporated herein by reference.

In direct labeling, the radionuclide binds to the functional group of amino acid side chains present in the peptide or protein. The radionuclide may also bind to reduced forms of a peptide or protein, such as a peptide or

protein containing a reduced disulfide bond. One example of direct labeling known in the art is described in U.S. Patent No. 4,877,868, which is incorporated herein by reference.

5                   Another well known technique for labeling peptides and proteins involves covalently binding the radionuclide to one specific functional group of an amino acid side chain, such as incorporation of iodide into the phenol group of a tyrosine residue.

10                   Commercial products for preparing radiopharmaceuticals are generally provided as lyophilized (freeze-dried) "kits" or as liquid formulations. Lyophilized kits are well known in the art. According to the present invention, lyophilized kits may contain a  
15                   transfer ligand, such as sodium gluconate or sodium tartrate, a reducing agent, such as tin, depending on the radioisotope that is used, a bulking agent, such as inositol or lactose, the peptide or protein to be labeled, and a surfactant as a stabilizer. Additional stabilizers,  
20                   such as suitable salts, may be incorporated into the formulation. A solution containing the radioisotope is added to the lyophilized kits prior to patient administration. In some cases, the reconstituted kit may need to be deoxygenated or may be heated or autoclaved.

25                   Liquid formulations usually contain the peptide or protein labeled with the radioisotope in a solution that is ready to be administered to the patient. According to the present invention, the liquid formulation contains a surfactant as a stabilizer. Additional stabilizers, such  
30                   as suitable salts, may be incorporated into the formulation. The liquid formulation may need to be deoxygenated or may be heated or autoclaved.

The radiolabeled compositions of the invention may be administered parenterally, preferably intravenously, in the form of injectable pharmaceutical solutions or suspensions according to conventional techniques.

5           Dosages employed in practicing the therapeutic method of the present invention will of course vary depending on the particular condition to be treated, for example the volume of the tumor, the particular chelate employed, the half-life of radioisotope, and the therapy  
10 desired. In general, the dose is calculated on the basis of radioactivity distribution to each organ and on observed target uptake.

          Due to the unique mechanism of the surfactants employed in the present invention to stabilize the  
15 radiolabeled peptide, polypeptide or protein compositions to avoid radiolysis thereof, (which may destroy the ability of the peptides, polypeptides or proteins to bind to specific biological receptors and may alter the biodistribution), the use of radiolabeled compounds for  
20 diagnostic imaging or radiotherapy of targeted tissues is now possible with significantly greater ease and success due to greater stability thereof.

          It is therefore an object of the present invention to provide a suitable surfactant for stabilizing radiolabeled  
25 peptides, polypeptides and proteins in diagnostic or therapeutic pharmaceutical preparations that are prepared at ambient temperature, or are heated or autoclaved, and are preferably stored at ambient temperature.

DETAILED DESCRIPTION OF THE INVENTION

The preferred surfactant employed in the present invention is polysorbate 80 (polyoxyethylene (20) sorbitan monooleate), also known as "Tween® 80", (registered trademark of Atlas Chemical Industries, Inc.) which is a non-ionic surfactant. Other non-ionic surfactants such as polyethyleneglycol p-isooctylphenylether may be used to stabilize the radiolabeled peptide, polypeptide, or protein compositions. Examples of other types of surfactants that could be used include, but are not limited to, cationic surfactants such as but not limited to cetyltrimethylammonium bromide, anionic surfactants such as but not limited to sodium dodecylsulfate, or zwitterionic surfactants such as but not limited to N-dodecylsultaine.

The advantages of adding one or more surfactants alone or in combination with one or more salts to radiolabeled peptide, polypeptide and protein compounds is to effectively prevent decomposition thereof from radiolysis. Suitable stabilizers combined with suitable salts have likewise been found to increase the stabilization of such radiolabeled compounds. Examples of such suitable salts include but are not limited to NaCl, KCl, CaCl<sub>2</sub>, and MgCl<sub>2</sub>. Studies show that the combination of the surfactant polysorbate 80 and NaCl led to improved stabilization of the radiolabeled compounds over that achieved by using polysorbate 80 alone. A summary of the results of this study is illustrated in Table 1 of Example 4 below.

The method of stabilizing radiolabeled compounds of the present invention is described in still greater detail in the illustrative examples which follow.

**EXAMPLE 1: Preparation of In-111 Labeled Peptide With No Additives.**

A stock solution was prepared by dissolving 56 mg trisodium citrate dihydrate, 4 mg citric acid monohydrate, 20 mg gentisic acid, and 100 mg inositol in 10 ml water. The pH was adjusted to 4.0 with 1.0 N HCl. The solution was purged for 30 minutes with argon.

A 10 microliter aliquot of a 1 mg DTPA-octreotide [N-(diethylenetriamine-N,N,N',N''-tetraacetic acid-N''-acetyl)-D-phenylalanyl-L-hemicystyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-L-hemicystyl-L-threoninol cyclic (2-7) disulfide] per 1 ml water solution was transferred into a 5 ml tubing vial and then 1 ml of the stock solution and 20 microliter of a In-111/0.05M HCl solution were added, yielding a solution containing 4.2 mCi/ml. A 0.5 ml aliquot of this preparation was transferred to another 5 ml tubing vial and stored at room temperature. The remaining 0.5 ml of the reaction solution was autoclaved for 15 minutes at 121°C.

The radiochemical yield of the In-111 DTPA-octreotide was determined using reverse phase HPLC with a mobile phase gradient. The column used was an Absorbosphere C<sub>18</sub> column, 5 micrometer, 260mm x 4.6mm. Mobile phase A contained 40:60 methanol:water, 50mM sodium acetate, pH 5.5. Mobile phase B contained 80:20 methanol:water, 50mM sodium acetate, pH 5.5. The gradient was a linear gradient from 100% A to 100% B over 20 minutes. The retention time of the In-111 DTPA-octreotide was between 19.4 - 19.8 minutes. Peptide decomposition products eluted prior to the In-111 DTPA-octreotide peak or eluted after the peak when the mobile phase reached 100% B.

The radiochemical purity of the room temperature preparation and the autoclaved preparation were measured by HPLC initially after preparation, after 3 days, and after 4 days. The RT preparation had 98%, 90%, and 86% radiochemical purity values and the autoclaved preparation had 93%, 89%, and 90% radiochemical purity values as illustrated in Table 1 of Example 4 below.

**EXAMPLE 2: Preparation of In-111 Labeled Peptide With Polysorbate 80.**

10 The procedure for this preparation was the same as for the preparation in Example 1 except that 20 microliters of 20% Polysorbate 80 in ethanol was added to the 1 ml solution of In-111 DTPA-octreotide. The vial contained 4.97 mCi/ml. The preparation was divided in half, with 0.5 ml kept at room temperature and 0.5 ml autoclaved for 15 minutes at 121°C. The radiochemical purity of the In-111 DTPA-octreotide was measured using the HPLC method described in Example 1.

20 The radiochemical purity for these samples initially after preparation, after 3 days, and after 4 days were measured by HPLC. The RT preparation + Polysorbate 80 had radiochemical purity values of 97%, 97%, and 97% and the autoclaved preparation + Polysorbate 80 had radiochemical purity values of 94%, 94%, and 92% as illustrated in Table 1 in Example below.

25 **EXAMPLE 3: Preparation of In-111 Labeled Peptide With NaCl.**

The procedure for this preparation was the same as for the preparation in Example 1 except that 7 mg NaCl was added to the 1 ml solution of In-111 DTPA-octreotide. The vial

contained 4.92 mCi/ml. The preparation was divided in half, with 0.5 ml kept at room temperature and 0.5 ml autoclaved for 15 minutes at 121°C. The radiochemical purity of the In-111 DTPA-octreotide was measured using the HPLC method described in Example 1.

The radiochemical purity for these samples after initial preparation, after 3 days, and after 4 days were 97%, 97%, and 97% for the RT preparation + NaCl and were 94%, 93%, and 91% for the autoclaved preparation + NaCl.

**10    EXAMPLE 4:    Preparation of In-111 Labeled Peptide With Polysorbate 80 and NaCl.**

The procedure for this preparation was the same as for the preparation in Example 1 except that 20 microliters of 20% Polysorbate 80 in ethanol and 7.0 mg of NaCl was added to the 1 ml solution of In-111 DTPA-octreotide. The vial contained 4.83 mCi/ml. The preparation was divided in half, with 0.5 ml kept at room temperature and 0.5 ml autoclaved for 15 minutes at 121°C. The radiochemical purity of the In-111 DTPA-octreotide was measured using the HPLC method described in Example 1.

The radiochemical purity for these samples initially after preparation, after 3 days, and after 4 days were 98%, 97%, and 97% for the RT preparation + Polysorbate 80 + NaCl and were 95%, 94%, and 93% for the autoclaved preparation + Polysorbate 80 + NaCl.

14

TABLE 1.: RADIOCHEMICAL PURITY VALUES FOR PREPARATIONS FROM  
EXAMPLES 1 THROUGH 4.

A. Radiochemical Purity of Room Temperature Preparations:

	<u>Initial</u>	<u>t=3d</u>	<u>t=4d</u>
5 RT	98%	90%	86%
RT + Polysorbate 80	97%	97%	97%
RT + NaCl	97%	97%	97%
RT + Polysorbate 80 + NaCl	98%	97%	97%

B. Radiochemical Purity of Autoclaved Preparations:

10

	<u>Initial</u>	<u>t=3d</u>	<u>t=4d</u>
Autoclaved	93%	89%	90%
Autoclaved + Polysorbate 80	94%	94%	92%
Autoclaved + NaCl	94%	93%	91%
15 Autoclaved + Polysorbate 80 + NaCl	95%	94%	93%



**EXAMPLE 5: Preparation of In-111 Labeled Peptide Without And With Polysorbate 80.**

A stock solution was prepared by dissolving 1.5 mg gentisic acid and 33.5 mg sodium gentisate in 10 ml water. The pH of the solution was 4.2. The solution was purged for 30 minutes with argon.

A 10 microliter aliquot of a 1 mg DTPA-octreotide [N-(diethylenetriamine-N,N,N',N''-tetraacetic acid-N''-acetyl)-D-phenylalanyl-L-hemicystyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-L-hemicystyl-L-threoninol cyclic (2-7) disulfide] per 1 ml water solution was transferred into a 5 ml tubing vial and then 1 ml of the stock solution and 20 microliters of an In-111/0.05M HCl solution were added, yielding a solution containing 5.65 mCi/ml. A 0.5 ml aliquot of this preparation was transferred to a 5 ml tubing vial and stored at room temperature. The remaining 0.5 ml was autoclaved for 15 minutes at 121°C.

The radiochemical purity of this preparation was determined by HPLC as described in Example 1.

A second preparation was made as described above except that 20 microliters of a 20% Polysorbate 80 solution in ethanol was added to the 1 ml reaction solution. To this solution was added 100 microliters of In-111/0.05M HCl, yielding a solution containing 5.9 mCi/ml. The solution was divided in half, and 0.5 ml of the solution in a 5 ml tubing vial was autoclaved for 15 minutes at 121°C.

16

The radiochemical purity for the autoclaved samples after initial preparation, after 1 day, and after 4 days is tabulated below.

Time	<u>Initial</u>	<u>t=3d</u>	<u>t=4d</u>
5 Autoclaved Preparation	91%	88%	79%
Autoclaved Preparation + Polysorbate 80	96%	95%	95%

After the radiolabeled peptide, polypeptide, or protein is stabilized according to the procedure described above, the compound is used with a pharmaceutically acceptable carrier in a method of performing a diagnostic imaging procedure using a scintillation camera or like device or a therapeutic treatment. This procedure involves injecting or administering, for example in the form of an injectable liquid, to a warm-blooded animal an effective amount of the present invention and then exposing the warm-blooded animal to an imaging procedure using a suitable detector, e.g. a scintillation camera. Images are obtained by recording emitted radiation of tissue or the pathological process in which the radioactive peptide, polypeptide, or protein has been incorporated, thereby imaging at least a portion of the body of the warm-blooded animal. Pharmaceutically acceptable carriers for either diagnostic or therapeutic use include those that are suitable for injection or administration such as aqueous buffer solutions, e.g. tris (hydroxymethyl)aminomethane (and its salts), phosphate, citrate, bicarbonate, etc.,

sterile water for injection, physiological saline, and balanced ionic solutions containing chloride and/or bicarbonate salts of normal blood plasma cations such as  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Mg}^{2+}$ . Other buffer solutions are described  
5 in Remington's Practice of Pharmacy, 11th edition, for example on page 170. The carriers may contain a chelating agent, e.g. a small amount of ethylenediaminetetraacetic acid, calcium disodium salt, or other pharmaceutically acceptable chelating agents.

10 The concentration of radiolabeled peptide, polypeptide, or protein and the pharmaceutically acceptable carrier, for example in an aqueous medium, varies with the particular field of use. A sufficient amount is present in the pharmaceutically acceptable carrier in the present  
15 invention when satisfactory visualization of the targeted tissue is achievable or therapeutic results are achievable.

The stabilized compositions of the present invention are administered to the warm-blooded animals so that the composition remains in the living animal for about  
20 six to seven hours, although shorter and longer residence periods are normally acceptable.

After consideration of the above specification, it will be appreciated that many improvements and modifications in the details may be made without departing  
25 from the spirit and scope of the invention. It is to be understood, therefore, that the invention is in no way limited, except as defined by the appended claims.

We claim:

1. A diagnostic composition suitable for administration to a warm-blooded animal comprising a peptide, polypeptide or protein labeled with a radionuclide optionally by means of a chelate and stabilized by one or more suitable surfactants to prevent radiolysis of said peptide, polypeptide, or protein and capable of administration to an animal to produce reliable diagnostic imaging thereof.
2. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an imaging-effective amount of a peptide, polypeptide or protein labeled with a radionuclide optionally by means of a chelate and stabilized by one or more suitable surfactants to prevent radiolysis, to allow for diagnostic imaging thereof.
3. The diagnostic composition of claim 1 wherein said one or more surfactants are selected from the group consisting of cationic, anionic, nonionic and zwitterionic surfactants.
4. The method of claim 2 wherein said one or more surfactants are selected from the group consisting of cationic, anionic, nonionic and zwitterionic surfactants.
5. A therapeutic composition suitable for administration to a warm-blooded animal comprising a peptide, polypeptide or protein labeled with a radionuclide optionally by means of a chelate and stabilized by one or more suitable surfactants to prevent radiolysis of said therapeutic composition and capable of administration to an animal to produce therapeutic effects on targeted tissues.
6. A method of performing a therapeutic procedure which

comprises administering to a warm-blooded animal a therapeutically-effective amount of a peptide, polypeptide or protein labeled with a radionuclide optionally by means of a chelate and stabilized by one or more suitable  
5 surfactants to prevent radiolysis of said composition and to allow for therapeutic effects on targeted tissues.

7. The therapeutic composition of claim 5, wherein said one or more surfactants are selected from the group consisting of cationic, anionic, nonionic and zwitterionic  
10 surfactants.

8. The method of claim 6, wherein said one or more surfactants are selected from the group consisting of cationic, anionic, nonionic and zwitterionic surfactants.

9. A diagnostic composition suitable for administration  
15 to a warm-blooded animal comprising a peptide, polypeptide or protein with receptor specificity labeled with a radionuclide optionally by means of a chelate and stabilized by one or more suitable surfactants and one or more suitable salts to prevent radiolysis of said  
20 composition, capable of administration to an animal to produce reliable diagnostic imaging thereof.

10. A method of performing a diagnostic procedure, which comprises administering to a warm-blooded animal an imaging-effective amount of a peptide, polypeptide or  
25 protein with receptor specificity labeled with a radionuclide optionally by means of a chelate and stabilized by one or more suitable surfactants and one or more suitable salts to prevent radiolysis of said peptide, polypeptide or protein, to allow for diagnostic imaging of  
30 targeted tissues.

11. The diagnostic composition of claim 9, wherein said one or more surfactants are selected from the group consisting of cationic, anionic, nonionic and zwitterionic surfactants.
- 5 12. The method of claim 10, wherein said one or more surfactants are selected from the group consisting of cationic, anionic, nonionic and zwitterionic surfactants.
13. The diagnostic composition of claim 9, wherein said one or more salts are selected from the group consisting of  
10 NaCl, KCl, NaCl<sub>2</sub>, and MgCl<sub>2</sub>.
14. The diagnostic composition of claim 9, wherein said one or more surfactants is polysorbate 80 and said one or more salts is NaCl.
- 15 15. The method of claim 10, wherein said one or more salts are selected from the group consisting of NaCl, KCl, NaCl<sub>2</sub>, and MgCl<sub>2</sub>.
16. The method of claim 10, wherein said one or more surfactants is polysorbate 80 and said one or more salts is NaCl.
- 20 17. A therapeutic composition suitable for administration to a warm-blooded animal comprising a peptide, polypeptide or protein with receptor specificity labeled with a radionuclide optionally by means of a chelate and  
25 more suitable salts to prevent radiolysis of said compositions, to provide therapeutic effects on targeted tissues.
18. A method of performing a therapeutic procedure, which

21

comprises administering to a warm-blooded animal a therapeutically-effective amount of a peptide, polypeptide or protein with receptor specificity labeled with a radionuclide optionally by means of a chelate and  
5 stabilized by one or more suitable surfactants and one or more suitable salts to prevent radiolysis of said composition, to provide therapeutic effects on targeted tissues.

19. The therapeutic composition of claim 17, wherein said  
10 one or more surfactants are selected from the group consisting of cationic, anionic, nonionic and zwitterionic surfactants.

20. The therapeutic composition of claim 17, wherein said one or more salts are selected from the group consisting of  
15 NaCl, KCl, NaCl<sub>2</sub>, and MgCl<sub>2</sub>.

21. The therapeutic composition of claim 17, wherein said one or more surfactants is polysorbate 80 and said one or more salts is NaCl.

22. The method of claim 18, wherein said one or more  
20 surfactants are selected from the group consisting of cationic, anionic, nonionic and zwitterionic surfactants.

23. The method of claim 18, wherein said one or more salts are selected from the group consisting of NaCl, KCl, NaCl<sub>2</sub>, and MgCl<sub>2</sub>.

24. The method of claim 18, wherein said one or more  
25 surfactants is polysorbate 80 and said one or more salts is NaCl.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US95/03180**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : C07K 7/00, 14/00, 16/00; C12P 21/08

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 178/1, 181.1, 179.1, 184.1; 415/1, 12, 8; 530/387.1, 388.1, 389.1, 391.3, 391.9

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, WPI, APS

SEARCH TERMS: SURFACTANT, TWEEN 80, ANTIBODY, DIAGNOSTIC, THERAPEUTIC, POLYOXYETHYLENE, SORBITAN, MONO-OLEATE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,853,330 (GOEDDEL ET AL.) 01 AUGUST 1989, SEE ENTIRE DOCUMENT.	1-24
Y	US, A, 4,479,930 (HNATOWICH) 30 OCTOBER 1984, SEE ENTIRE DOCUMENT	1-24
Y	US, A, 5,080,883 (LYLE ET AL.) 14 JANUARY 1992, SEE ENTIRE DOCUMENT	1-24
Y	IMMUNOLOGY TODAY, VOLUME 11, NUMBER 6, ISSUED 1990, OSBAND ET AL., "PROBLEMS IN THE INVESTIGATIONAL STUDY AND CLINICAL USE OF CANCER IMMUNOTHERAPY" PAGES 193-195, SEE ENTIRE DOCUMENT	1-24

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A document defining the general state of the art which is not considered to be of particular relevance	*X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E earlier document published on or after the international filing date	*Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z	document member of the same patent family
*O document referring to an oral disclosure, use, exhibition or other means		
*P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

15 MAY 1995

Date of mailing of the international search report

31 MAY 1995

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

DONALD E. ADAMS, PH.D.

Telephone No. (703) 308-0196



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US95/03180

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AM. J. PHYSIOLOGY, VOLUME 232, NUMBER 3, ISSUED MARCH 1977, MILLARD ET AL., "CARDIOVASCULAR EFFECTS OF RADIOACTIVE MICROSPHERE SUSPENSIONS AND TWEEN 80 SOLUTIONS," PAGES H331-H334, SEE ENTIRE DOCUMENT.	1-24
Y	HIRD ET AL. , "GENES AND CANCER" PUBLISHED 1990 BY JOHN WILEY & SONS, LTD.(ENGLAND), PAGES 183-189, SEE ENTIRE DOCUMENT.	1-24
Y	TIBTECH, VOLUME 11, ISSUED 1993, HARRIS ET AL., "THERAPEUTIC ANTIBODIES- THE COMING OF AGE," PAGES 42-46, SEE ENTIRE DOCUMENT.	1-24
Y	ANN. INTERNAL MEDICINE, VOLUME 111, ISSUED 1989, DILLMAN, "MONOCLONAL ANTIBODIES FOR TREATING CANCER," PAGES 592-603, SEE ENTIRE DOCUMENT.	1-24
Y	SCIENCE, VOLUME 252, ISSUED 21 JUNE 1991, WALDMANN, "MONOCLONAL ANTIBODIES IN DIAGNOSIS AND THERAPY," PAGES 1657-1662, SEE ENTIRE DOCUMENT.	1-24
Y	HUSA'S "PHARMACEUTICAL DISPENSING." PUBLISHED 1966 BY MACK PUBLISHING COMPANY, (PA), PAGES 177-180, SEE ENTIRE DOCUMENT.	1-24

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US95/03180

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

424/130.1, 178/1, 181.1, 179.1, 184.1; 415/1, 12, 8; 530/387.1, 388.1, 389.1, 391.3, 391.9